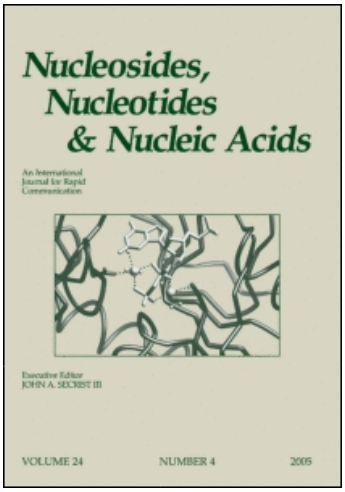

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PHOSPHONATE ANALOGUES OF PEPTIDE NUCLEIC ACIDS AND RELATED COMPOUNDS: SYNTHESIS AND HYBRIDIZATION PROPERTIES

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ABSTRACT. The synthesis of a set of novel chimeric oligomers representing PNA and phosphono-PNA analogues has been accomplished, and their binding affinity to complementary DNA and RNA strands was evaluated.

With the aim to improve physico-chemical and biological properties of peptide nucleic acids (PNAs), particularly water solubility and cellular uptake, we have undertaken the investigations on the design and synthesis of related DNA mimics. Recently, chimeras representing phosphonate analogues of PNAs (pPNAs) and PNA-pPNA hybrids containing the four natural nucleobases have been reported[1-3]. The simple procedures for the preparation of PNA[4] and pPNA[3] monomers as well as PNA-pPNA dimers were developed, which were used in the efficient automated solid phase synthesis of corresponding oligomers[3,4]. A set of oligomers representing optically active pPNA analogues as well as hybrids containing monomers with natural nucleobases replaced by non-canonical bases (7,8-dimethylalloxazine or 2,6-diaminopurine) and fluorescent dyes (pyrene or 4-nitro-1,8-naphthalimide) embedded into a mimic chain was prepared [4]. The properties of the oligonucleotide mimics obtained were examined. It was found that, along with good solubility in water, the chimeras obtained form stable complexes with complementary DNA and RNA fragments, and the PNA-pPNA hybrids are the most promising for further evaluation as potential antisense and antigene agents. In this paper, we describe the extension of these investigations to the development of a set of novel PNA- and pPNA-related molecules and hybrids (Fig. 1).

Among these novel mimics, hetero-oligomers consisting of various amounts of pPNA monomers and 4-hydroxyproline (Hyp) residues have been synthesized by solid phase technique. The dimer building blocks (1a,b) has been constructed and incorporated into a pPNA.chain using protocols for the phosphonate ester and amide bond formation developed by us earlier[3,4]. Similarly, the automated synthesis of pPNA-Ser chimeras has been accomplished using the corresponding dimers (2a,b)[5] (Fig.2). The examination of hybridization properties of these chimeras to DNA and RNA complementary strands revealed that pPNA-Hyp chimeras show stronger binding to complementary DNA and RNA strands than the original pure pPNAs

and equivalent PNA-pPNA hybrids. The best results were obtained for oligomers with alternating pPNA and Hyp residues (Table 1). The stability of pPNA-Hyp / DNA (RNA) complexes was very close to that for PNA/DNA(RNA) complexes. However, pPNA-Ser hybrids gave less stable complexes with

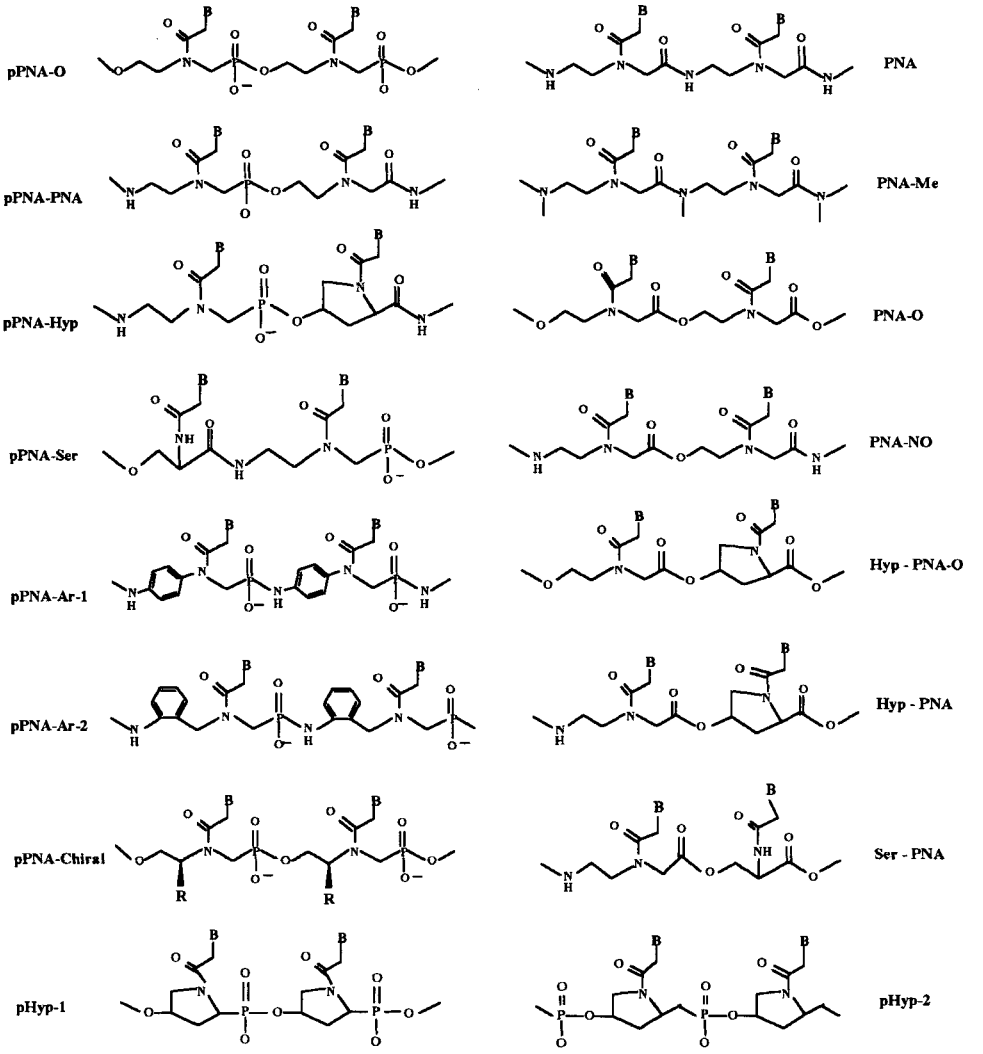

FIGURE 1. The structures of the PNA and pPNA analogues and hybrids obtained in this study.

oligo-dA (rA) with melting temperatures close to those of natural oligo-T/oligo-A duplexes. From the titration data, it can be concluded that both pPNA-Hyp or pPNA-Ser homo-pyrimidine sequences form with poly-A templates triple helixes, whereas sequences containing all four nucleobases gave duplexes. The pPNA-Hyp hybrids are consequently potential candidates as antisense compounds for applications in therapy and diagnostics.

Moreover, two types of pPNA-like oligomers on the base of 4-hydroxyproline (pHyp-1,2) as well as two aromatic pPNA analogues (pPNA-Ar-1,2) were prepared (Fig. 1). The synthesis of the

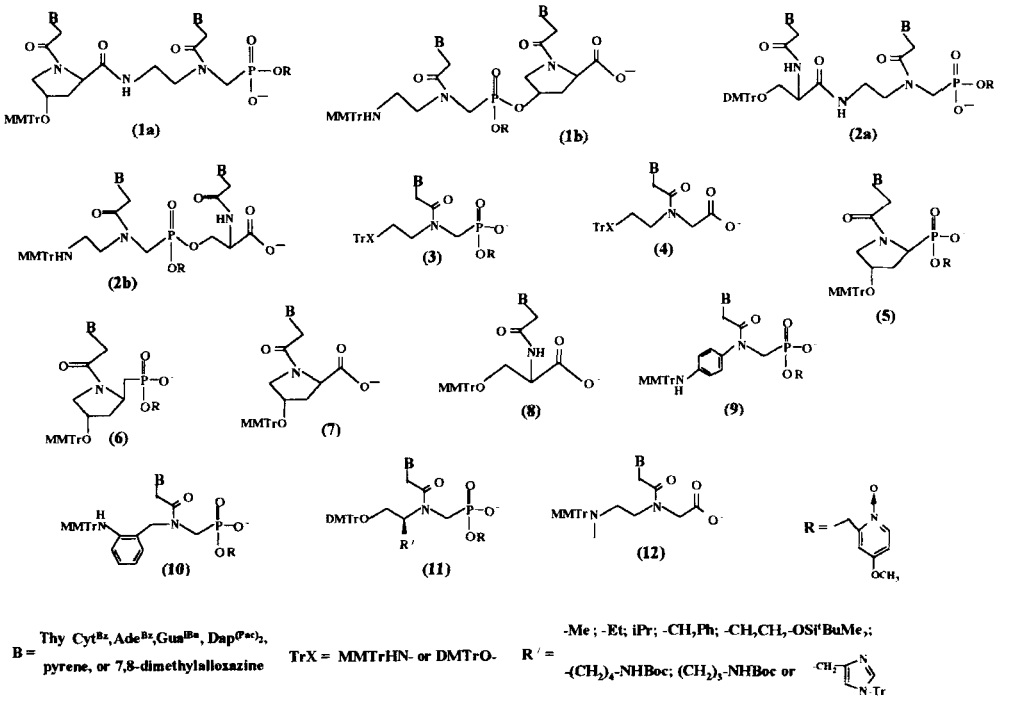

FIGURE 2. Chemical structures of monomers used for the synthesis of the mimics obtained.

corresponding monomers (5,6) and (9,10) (Fig.2) has been accomplished using the routes similar to those developed by us earlier for the preparation of standard pPNA monomers with N-(2-aminoethyl)phosphonoglycine backbone[3]. These monomers were used in the automated synthesis of oligo-Thy sequences using CPG support and protocols for the phosphonester (phosphonamide) bond formation[3,4]. In parallel, we synthesized several pPNA analogues starting from optically active monomers of type (11) containing various natural amino acids side chains incorporated in the same position of N-(2-hydroxyethyl)phosphonoglycine backbone. The physico-chemical properties of these chiral pPNA oligomers are currently under investigation.

The next set of mimics obtained in this study was represented modified homo-Thy PNA oligomers, particularly N-(2-methylaminoethyl)glycine (PNA-Me) and N-(2-hydroxyethyl)glycine (PNA-O) derivatives as well as hetero-PNAs containing alternated amide and ester bonds between monomers (PNA-NO) (Fig. 1). Similarly, hetero-oligomers with alternating 4-hydroxyproline and PNA residues (Hyp-PNA and Hyp-PNA-O), or serine and PNA monomers (Ser-PNA) have been constructed. The solid phase synthesis of N-methylated PNA analogues was accomplished using the monomer (12) and a protocol developed by us earlier for the synthesis of regular PNAs [3,4]. The automated synthesis of polyester analogues was performed starting from monomers (4), (7) and (8) on an alkylsulfonylethyl-CPG support[6], which was functionalized with p-chlorophenyl ester of 5'-

TABLE 1. Melting temperatures of complexes formed by homo-Thy_{15} oligomers with dA_{15} or rA_{15} targets in 0.15 M NaCl / 20 mM Tris-HCl / 5 mM $MgCl_2$ (pH 7). The values were measured at 260 nm using 3 μM concentrations of oligomers and heating from 2°C to 95°C (0.5°C/min).

Oligomer type	T*m* (Δ T*m*),°C / DNA-target	T*m* (Δ T*m*), °C / RNA-target
DNA	42	40
pPNA-O	49 (+7)	28 (-12)
pPNA-PNA (1:1)	65 (+23)	57 (+17)
pPNA-Hyp (1:1)	83 (+41)	77 (+37)
pPNA-Ser (1:1)	40 (-2)	36 (-4)
PNA	85 (+43)	82 (+42)
PNA-Me	55 (+13)	49 (+9)
PNA-O	60 (+18)	57 (+17)
PNA-N,O (1:1)	72 (+30)	68 (+28)
Hyp-PNA-O (1:1)	65 (+23)	62 (+22)
Hyp-PNA (1:1)	81 (+39)	78 (+38)
Ser-PNA (1:1)	56 (+14)	53 (+13)

dimethoxytritylthymidine 3'-phosphate. The synthetic cycle for construction of polyester chains was similar to that for the synthesis of pPNA oligomers[3] . On each elongation step, TPSCl in the presence of MeIm was used as a condensing agent for the formation of ester bond between carboxyl moiety of a monomer unit and a terminal hydroxyl group on the support. The condensations were performed in acetonitrile - pyridine mixture for 10 min. After the cleavage from the support by the action of *tert*-butylamine and removal of terminal dimethoxy- or monometoxytrityl group by the action of 80% acetic acid, polyester PNA analogues were isolated by RP-FPLC. The investigation of hybridization properties of these homo-pyrimidine polyester oligomers revealed that as well as regular PNAs, they form stable triplexes with the complementary DNA, or RNA. Among these polyester analogues, the highest Tm values were shown by Hyp-PNA and Hyp-PNA-O hybrids (Table 1).

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